partially protrude it, and quickly pull it back, this continuing for four or five or more times before he could hold it out. The face could not be drawn up at the side voluntarily by either brother.

The deep reflexes were exaggerated but no Babinski. No sensory changes, either objective or subjective.

Finer movements, as in eating, etc., were impossible or most difficult.

Ataxia in knee-, heel-, and finger-to-nose tests was well marked. The patient could neither sit nor stand quietly. In this respect both patients resembled very much cases of Huntington's Chorea. Had incontinence of urine four or five times about ten years ago. He says his sexual power is not impaired.

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# CHOLESTERIN ANTIGENS IN THE WASSERMANN REACTION AND THE QUANTITATIVE TESTING OF SYPHILITIC SERA.

BY J. G. HOPKINS, M.D.
ASSOCIATE IN BACTERIOLOGY, COLUMBIA UNIVERSITY,
AND
J. B. ZIMMERMANN.

(From the Pathological Department of St. Luke's Hospital, New York; F. C. Wood, M.D., Director.)

1. CHOLESTERIN ANTIGENS. The addition of cholesterin to lecithin solutions or to alcoholic organ extracts used as "antigens" in the Wassermann reaction was first recommended by Browning, Cruickshank, and M'Kenzie, and later by Sachs, Walker and Swift, and others. The experience of all has been that such preparations give fixation of complement with many syphilitic sera which fail to react with similar extracts without the cholesterin. Walker and Swift tested the sera of twenty-two individuals in whom syphilis could reasonably be excluded with cholesterin-heart extract preparations and obtained no fixation except when an excessive amount of the extract was used, in which case some of the sera gave partial fixation. Recently, Thomas and Ivy have reported a number of false positive reactions obtained with these antigens. It should be noted, however, that they used only the minimal hemolytic dose of amboceptor and complement, a practice which of itself makes the test extremely sensitive and also unreliable, according to the experience of most workers.

The advantage of using antigens which will increase the number of positive reactions is obvious, providing there is reasonable certainty that the antigens will not react with non-syphilitic serum. However, some procedures which increase the delicacy of the reaction, such as testing the serum without previous inactivation or using minimal amounts of complement and amboceptor, have been found to give non-specific reactions, and it seemed possible that such might be the case with the cholesterin antigens. For this reason we determined to test their sensitiveness and reliability by using them in a series of routine examinations, testing the same sera simultaneously with extracts possessing known properties.

The Wassermann reactions were carried out with one-half the original amounts: that is, 0.1 c.c. of patient's serum, 0.5 c.c. of diluted antigen, and usually 0.05 c.c. of guinea-pig serum (the exact amount being determined each day by preliminary titration) in the first stage, and 1 c.c. of 25 per cent. sheep cells sensitized with twice the minimal hemolytic dose of immune rabbit serum in the second stage. In testing spinal fluids 0.5 c.c. was used. Controls for anticomplementary action, one containing double the amount of serum (or spinal fluid) and one containing double the amount of antigen used in the test were always made. When the controls showed any inhibition the tests were disregarded.

The extracts were prepared by treating chopped moist guinea-pig heart with absolute alcohol for two to three weeks at 37° C., the proportions being 1 gm. of tissue to 10 c.c. of alcohol. To the filtered extract 0.4 per cent. cholesterin was added, and after this had stood for twenty-four hours in the incubator the undissolved residue was filtered off. This method is that recommended by Walker and Swift, except that guinea-pig heart was used instead of human. Emulsions for use in the tests were prepared by adding salt solution, at first drop by drop and then rapidly, to the alcoholic extract, the mixture being shaken during the process. A few tests were made with emulsions prepared in the reverse manner, the alcoholic extract being dropped into the salt solution, but the results seemed less satisfactory. Sachs and Rondoni found, in regard to simple alcoholic extracts, that dilutions made in the latter way were less anticomplementary but proportionately still less active as antigens, and consequently less satisfactory than the more turbid emulsions produced by the slow addition of salt solution to the extract. This has also been our own experience. The anticomplementary power of all the extracts was determined before use, and it was found that most of them produced slight inhibition in amounts from 0.1 to 0.12 c.c., but none in amounts of 0.08 c.c. or less. In performing the reactions one-fourth of the largest amount which showed no inhibition was used—usually 0.02 or 0.025 c.c.

In the control series of tests the acetone insoluble lipoids of beef-heart extract, prepared by Noguchi's method, were used as antigen.

The reactions obtained with sera from 475 patients and with 50 specimens of spinal fluid are shown in Table I.

TABLE I.

| Condition. | Number of cases. | Lipoid antigen. Number of positive reactions. | Cholesterin antigen. Number of positive reactions. |
|---|---|---|---|
| Blood-serum: | | | |
| Primary syphilis | 10 | 6 | 7 |
| Secondary syphilis | 25 | 21 | 25 |
| Tertiary syphilis | 99 | 59 | 79 |
| Congenital syphilis | 9 | 5 | 7 |
| Tabes dorsalis | 20 | 9 | 13 |
| General paresis | 4 | 4 | 4 |
| Latent or cured syphilis | 44 | 17 | 29 |
| Apparently non-syphilitic | 264 | 4 | 25 |
| Spinal fluid: | | | |
| Tabes dorsalis | 19 | 8 | 10 |
| General paresis | 3 | 3 | 3 |
| Tertiary syphilis | 9 | 3 | 2 |
| Non-syphilitic | 19 | 0 | 1 |

In tabulating our results we have recorded as positive only those of the tests which showed no or very slight hemolysis after an hour's incubation in the second stage. When using the lipoid antigens all tests showing 50 per cent. or less hemolysis may safely be called positive, and such readings would give a somewhat larger proportion of positives with these antigens in luetic cases than the table shows. Many non-luetic sera, however, gave partial inhibition of hemolysis with the cholesterin-heart extract preparations, and better results were obtained with these if only those sera which caused practically complete inhibition were regarded as positive.

The cases are grouped in the table according to the clinical diagnosis. Many cases which seemed doubtful have been disregarded; but, even so, the probability of some errors in diagnosis must be considered before conclusions are drawn from these figures. One difficulty is that the diagnosis in many cases was doubtless influenced by the result of the Wassermann reaction. As a matter of fact, cases giving an indefinite history of lues with a positive serum reaction have been included under the head of "latent syphilis," whereas cases giving a negative reaction were not considered to be syphilitic unless the history or clinical picture was fairly characteristic of the disease.

Of the 525 reactions which have been included in the series the result was the same with both types of antigen in 455. Only two specimens which were positive with the "lipoid" antigen were negative with the "cholesterin." These were both spinal fluids, one from a case of tabes and one from a case of cerebrospinal syphilis. On the other hand, 68 specimens reacted positively with the "cholesterin" antigen, but not with the "lipoid;" of these cases 46 were syphilitic and 22 apparently non-syphilitic. Of the former, 3 were treated secondary cases and 1 an untreated secondary; but the majority were tertiary or latent cases. Of the 26 which have been classed as non-syphilitic, 1 was a case of sarcoma, 4 were cases of carcinoma, and 4 cases of pernicious anemia. One was a case of estivo-autumnal malaria, a condition in which positive Wassermanns have frequently been reported. The other cases represented a variety of conditions, many of them local infections; but except for the cases mentioned these false positive reactions were not found twice in the same disease, and there seems no reason to believe that the illness of the patient at the time was responsible for the result of the test. The Wassermann reactions were not made as a routine on all admissions, so that in nearly all these cases which we have classed as probably non-syphilitic the possibility of lues was considered at the time the tests were made. In several instances, however, the nature of the suspected gumma or ulcer was determined by histological examination; and in the remainder, further observation ruled out syphilis so far as the present illness was concerned. It is, however, impossible absolutely to exclude latent syphilis in these cases. Four cases that were clinically non-luetic gave reactions with both types of extracts used. It is certain that the "lipoid" antigens rarely, if ever, give false positive reactions, and it is probable, therefore, that these patients had unrecognized infections. It seems highly improbable, however, that 25 out of 264 patients, or 9 per cent., presenting no history or signs of syphilis, should in fact be luetic.

A factor which probably accounts for some of our non-specific reactions is the instability of these extracts. A number of the false positive tests were obtained with a preparation which was over six weeks old. At this time it was found that the anticomplementary power had increased very considerably, and although the amount used in the reaction was proportionately decreased, a number of positive tests which could not be explained by the clinical data were obtained. In view of this experience, antigens less than four weeks old only were subsequently used, with considerable improvement in the results. As the presence of a slight excess of antigen also seems to produce non-specific reactions the making of an anticomplementary control containing four times instead of twice the amount of antigen used in the test is recommended.

In spite of the apparently false reactions obtained, we believe that these extracts are of distinct value on account of the large number of positive results obtained in tertiary syphilis and in cases cured so far as symptoms were concerned, which failed to react with the "lipoid" antigens. There are obvious advantages in so sensitive a test in controlling treatment, or in cases where it is desired to exclude syphilis so far as possible, as, for example, in selecting donors for blood transfusion or in examining wet-nurses. The reactions are also very helpful in diagnosis when there is some clinical evidence in their support.

It should be remembered, however, that the nature of the Wassermann reaction is not known and that it is essentially empirical.

Until it can be shown by long experience, therefore, that these extracts do not react with non-luetic sera they should not replace extracts whose reliability has been established. What evidence we have been able to obtain indicates that they do give complement fixation with some non-syphilitic sera. It would seem advisable, then, in routine work to test all sera both with a sensitive cholesterin preparation and with some less sensitive but thoroughly reliable extract.

2. The Quantitative Testing of Syphilitic Sera. Another phase of the Wassermann technique which has interested us has been the determination of the strength of the reaction in positive sera. This is specially important in following cases under treatment. The most logical method is to make tests with various amounts of the serum, to determine how small an amount will produce the reaction. In order to measure the small amounts required at least two preliminary dilutions of each serum to be tested must be made, and this very considerably increases the labor of carrying out a large series of reactions. In some laboratories this difficulty is avoided by testing constant amounts of serum against serial dilutions of the antigen, which can be prepared in bulk for all the tests to be made on a given day.

A technique based on this principle was introduced by Coca and l'Esperance, and in their hands gave excellent results, and we have followed their procedure with slight variations in making some five hundred Wassermann tests. The reaction is carried out with one-tenth the volume used in the original Wassermann method. The patient's serum (unheated) is first diluted with four parts of salt solution, and 0.1 c.c. is placed in each of five tubes; in a sixth tube 0.2 c.c. is placed for a control. As antigen a 2 per cent. solution of the acetone insoluble fraction from normal beef-heart extract prepared by Noguchi's method is used. Emulsions are prepared containing 0.05, 0.025, 0.012, 0.006, and 0.003 c.c. of this stock solution per cubic centimeter, and each serum is tested with 0.1 c.c. of each of these dilutions; $\frac{1}{10}$ c.c. of a 1 in 10 dilution of guinea-pig serum is used in the test, with 0.1 c.c. of a 5 per cent. emulsion of sheep corpuscles and two units of hemolytic amboceptor.

The use of the patient's serum without previous inactivation is thought by some workers to be a safe procedure, provided that antigen free from protein constituents is used, although it is admitted that false positives are obtained with simple alcoholic organ extracts. Although in our tests a carefully prepared "lipoid" antigen was used, positive reactions were obtained in a few cases in which there was no evidence of syphilis other than this reaction, and in which the inactivated sera were negative both with "lipoid" and with "chloesterin" antigen. While some sera from tertiary and latent cases reacted positively in fresh condition, but not when inactivated, four sera which when active gave negative results, after inactivation

TABLE II.—Estimates of Strength of Sera Obtained by Various Methods, Compared.[1]

| | (a) Sera tested in graded amounts against 0.02 c.c. of lipoid antigen. | | | | | (b) Sera tested in graded amounts against 0.05 c.c. of cholesterin antigen. | | | | | (c) Same sera tested in constant amounts (0.1 c.c.) against graded amounts of lipoid antigen. | | | | | (d) Same sera tested in constant amounts (0 1 c.c.) against graded amounts of cholesterin antigen. | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Amount of patient's serum. | | | | | Amount of patient's serum. | | | | | Amount of antigen. | | | | | Amount of antigen. | | | | |
| | .1 | .05 | .025 | .0125 | .0062 | .1 | .05 | .025 | .0125 | .0062 | .02 | .01 | .005 | .0025 | .00125 | .05 | .025 | .0125 | .0062 | .0031 |
| 1. Secondary | 0 | 0 | 0 | ++ | ++++ | 0 | 0 | 0 | +++ | ++++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2. Tertiary | ++ | ++ | ++++ | ++++ | ++++ | 0 | 0 | + | ++++ | ++++ | ++ | ++++ | ++++ | ++++ | ++++ | 0 | 0 | 0 | 0 | 0 |
| 3. Tertiary | 0 | 0 | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + | ++ | +++ | 0 | 0 | 0 | 0 | + |
| 4. Tabes | 0 | 0 | 0 | + | ++++ | 0 | 0 | 0 | ++++ | ++++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5. Secondary | 0 | 0 | 0 | +++ | ++++ | 0 | 0 | + | ++++ | ++++ | – | 0 | 0 | 0 | – | 0 | 0 | 0 | 0 | 0 |
| 6. Latent | 0 | 0 | 0 | ++ | ++++ | 0 | 0 | +++ | ++++ | ++++ | 0 | 0 | 0 | +++ | ++++ | 0 | 0 | 0 | 0 | 0 |
| 7. Tertiary | 0 | 0 | 0 | ++ | ++++ | 0 | 0 | 0 | + | ++++ | 0 | 0 | 0 | 0 | ++ | 0 | 0 | 0 | 0 | 0 |
| 8. | 0 | + | ++++ | ++++ | ++++ | 0 | 0 | +++ | ++++ | ++++ | 0 | 0 | + | ++++ | ++++ | 0 | 0 | 0 | 0 | 0 |
| 9. Latent | 0 | 0 | 0 | ++ | ++++ | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | 0 | | | | | |
| 10. Latent | 0 | 0 | 0 | 0 | 0 | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | 0 | | | | | |
| 11. Latent | 0 | 0 | 0 | 0 | + | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | 0 | | | | | |
| 12. Tertiary | 0 | 0 | 0 | 0 | + | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | 0 | | | | | |
| 13. Tertiary | 0 | 0 | ++++ | ++++ | ++++ | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | ++++ | | | | | |
| 14. Tertiary | 0 | 0 | 0 | ++ | ++++ | .. | .. | .. | .... | .... | 0 | 0 | 0 | 0 | 0 | | | | | |

0 = no hemolysis.

++++ = complete hemolysis.

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[1] The cholesterin antigens in this experiment were in clear emulsion, obtained by adding the extract to the diluent drop by drop. Consequently, relatively large amounts were used.

gave positive results with the same antigen. These negative reactions were probably due to the complement in the patient's serum. The results make the employment of active serum appear distinctly disadvantageous. Moreover the small quantities used in this technique introduce the possibility of error on account of the care required to deliver 0.1 c.c. accurately from the blunt-tipped pipettes which are usually employed in this work.

The method was chiefly interesting, however, as a means of determining the strength of the reaction. The majority of the sera which reacted at all gave complete inhibition in at least four of the five tubes set up. For example in tertiary cases, where weaker reactions would be expected, out of 60 positive sera, 27 reacted in the fifth tube containing one-sixteenth of the usual amount of antigen, and 41 reacted in the fourth tube of the series. As so many specimens reacted alike the method did not give a very satisfactory grading of their strength. A series of higher dilutions would doubtless have brought out further differences. It has been asserted by MacIntosh and Fildes that titration with varying amounts of antigen does not give an accurate estimation of the strength of the reaction. They base this assertion on experiments in which the same sera were titrated, using varying amounts of serum with constant amounts of anitgen, and also using constant amounts of serum with varying amounts of antigen. We tested in this way fourteen sera, using the "lipoid" type of antigen, and eight with the "cholesterin" type also, as shown in Table II.

The titrations in which the amount of serum was graded (*a* and *b*, Table II) with both antigens showed considerable variations in strength among the sera used. When titrated in the reverse way with the less sensitive antigen (*c*) the relative values did not run parallel, and the differences between them were much obscured; while with the more sensitive antigen (*d*) these differences were lost almost altogether.

It seems then that a closer estimate of the strength of syphilitic serum may be obtained by determining how small a quantity of it will produce a reaction, rather than by determining with how small an amount of antigen it will react; or, in other words, that quantitative Wassermann tests should be made by varying the amount of serum used, and not the amount of antigen.

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